REMARKS

The Invention

The invention features methods and compositions for treating a subject with, or at risk of having, a Japanese cedar allergy.

Status of the Claims

Claims 16 and 17 are pending and under consideration in this application, claims 1-15 having been cancelled without prejudice to their being pursued in a separate application. Both pending claims stand rejected.

Amendments

The amendment to the specification on page 12, line 4 is supported by the full sentence spanning lines 1-5 on page 12; it is clear from its context that the word "antigen" on page 12, line 4, should be the word "antibody".

The amendment to the specification on page 28, line 24 (changing "180" to "80"), is supported by, for example, the correct designation of the relevant peptide together with its appropriate SEQ ID NO (i.e., SEQ ID NO:14) at multiple sites in the specification, e.g., at page 28, line 20, and page 29, lines 2-3, line 4, line 19, and line 23.

The amendment on page 13, line 15, is supported by a comparison of the sequences of the peptides composed of residues 211-225 and residues 221-235 (see Fig. 1) with SEQ ID NO:8 in the Sequence Listing; from such a comparison it is clear that the peptide referred to as "[p]221-225 SEQ ID NO: 8" on page 13, line 15 is actually the peptide composed of residues 211-225. No p221-225 is referred to anywhere else in the application or is listed in Fig. 1. Furthermore, such a peptide would contain only five amino acids versus the 15 amino acids of all the other peptides listed in Fig. 1.

The amendment on page 17, line 19, serves to correctly recite DPA1*0101/0101-DPB1*0501/0201 rather than DPB1*0101/0101-DPB1*0501/0201 as it was depicted prior to the amendment (see page 3, lines 9-15, of the specification). The correctness of the amended haplotype depiction is confirmed by the depiction of the same HLA class II haplotype in the text (page 23, lines 23-25) of Example 6 and the results obtained with

T cell clones from patient B (PB) in Figs. 3 and 4 referred to immediately prior to the incorrect depiction of the haplotype on page 17, line 19.

The grammar of the text on page 28, lines 19-21, is corrected. In addition, the number of overlapping peptides tested in the experiment described in this text is amended from 90 to 74. This amendment is supported by methodological description of the relevant experiment on the same page (page 28), lines 4-5; furthermore, Fig. 2 indicates that only 74 of the 15-mer peptides spanned the whole Cry j 2 polypeptide. This text is also amended to correct the SEQ ID NO assigned to p236-250 of Cry j 2 from SEQ ID NO:48 to SEQ ID NO:19 (see following paragraph).

The corrections to the text on page 28, line 24, page 29, line 26 to page 30, line 1, page 30, line 2, page 30, line 17, and page 30, line 21 serve to correct errors arising from a confusion between the original experimental number (48) assigned to a peptide (p236-250) and a subsequent SEQ ID NO (19) assigned to it during the drafting of the instant application. Thus, after amendment, the text referred to above now correctly refers to the peptide as either peptide no. 48 and SEQ ID NO:19 or as SEQ ID NO:19 alone. Support for these amendments is in Fig. 2 (peptide 236-250), in which the peptide is the 48th in the list (see also Fig. 6 which refers to peptide 236-250 as "peptide # 48") and SEQ ID NO:19 in the Sequence Listing.

Multiple occurrences of the term "patient" in claims 16 and 17 have been replaced with the term "subject." These amendments are supported by the specification, e.g., the paragraph spanning pages 16 and 17.

Additional amendments to the specification and the claims are described where appropriate below.

Amendments to the drawings serve to conform them to the Sequence Listing submitted April 10, 2001.

Amendments not specifically mentioned serve to conform the language to conventional English usage and to correct grammatic and typographic errors.

No new matter has been added by any of the amendments made herein.

Priority Claim

In response to the request in paragraph 3 on page 2 of the Office Action, Applicants have inserted a claim for priority as the first line of the specification.

Formal Drawings

As requested in paragraph 4 on page 2 of the Office Action, Applicants will submit formal drawings subsequent to receipt of a Notice of Allowance. The Examiner confirmed in a telephone conversation with Applicants' undersigned representative on July 8, 2002, that corrected drawings were not required to be submitted in response to the instant Office Action.

Amendment of a typographic error in claims 16 and 17

As requested in paragraph 5 on page 2 of the Office Action, Applicants have changed "DQB1*0501" in claims 16 and 17 to "DPB1*0501".

Amendment of a typographic error in the specification

As requested in paragraph 6 on page 2 of the Office Action, Applicants have changed "IF-Nγ" on page 27, line 8, of the specification to "IFN-γ". This amendment merely corrects a typographic error and adds no new matter.

35 U.S.C. §112, first paragraph, rejections

Claims 16 and 17 stand rejected on the grounds that: (a) the specification allegedly does not enable any person skilled in the art to which it pertains, or with which it is mostly nearly connected, to make and use the invention commensurate in scope with these claims; and (b) they allegedly contain subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention.

From the comments on pages 2-7 of the Office Action, Applicants understand the Examiner's position to be that the specification does not provide enablement or written

description for:

(1)"preventing" cedar pollen allergy;

(2) "treating" cedar pollen allergy with an antigen peptide having **SEQ ID NO: 25** if the HLA class II molecule identified is DQA1*0102-DQB1*0602;

(3) "treating" cedar pollen allergy with an antigen peptide having **SEQ ID NO: 12** if the HLA class II molecule identified is DRB4*0101; and

(4) "treating" cedar pollen allergy with an antigen peptide having SEQ ID NO: 22 if the HLA class II molecule identified is **DPA1*0202-DQB1*0501**.

In regard to rejections covered by item (1), the Examiner asserts that there is insufficient guidance and working examples using any peptide claimed for "preventing" (i.e., averting or keeping from happening) cedar pollen allergy. Applicants respectfully disagree with this assertion. The "preventive" effect is adequately supported by Examples 11 and 12. The T cells of the cedar pollen allergy model CB6F1 created in Example 10 recognize p66-80 (SEQ ID NO: 14) and p236-250 (SEQ ID NO: 19), which are two major T cell epitopes of cedar pollen allergy patients. The specification shows that when these epitopes were separately administered to CB6F1 mice prior to a challenge with recombinant Cry j2 allergen, the responsiveness (proliferation) of Cry j 2-specific T cells was significantly suppressed compared to controls (see Examples 11 and 12). These results thus show the *in vivo* "preventive" activity of two exemplary peptides. Applicants submit that, in light of this teaching, one of skill in the art would consider it likely that these two peptides, and the others listed in the claims, would be similarly immunosuppressive when administered to individuals expressing the appropriate MHC class II molecules. Moreover, written description for "prevention" is provided in the specification, e.g., at page 29, lines 22-25, and page 30, lines 20-24.

The rejections covered by items (2) - (4) above arise from typographical errors in the claims that are corrected by amendments made herein.

Specifically in regard to item (2), Applicants have deleted the antigen peptide with SEQ ID NO:25 from the claims and replaced it with p346-360 (SEQ ID:142) of Cry j 2. As can be seen from Fig. 4, antigen peptide p346-360 of Cry j 2 does indeed bind to the relevant HLA class II molecule (DQA1*0102-DQB1*0602). Moreover, as indicated

by a comparison of the amino acid sequence for this antigen peptide in Fig. 2 and the Sequence Listing filed December 7, 2001, it has the sequence identifier SEQ ID NO:142. Accordingly, the specification at page 13, line 15, is also appropriately amended. No new matter is added by these amendments that are not intended to, and do not, narrow the scope of the claims.

With respect to item (3), Fig. 4 shows that p16-30 (SEQ ID NO:12) does bind to DRB4*0101. Accordingly, the specification at page 13, line 7, is appropriately amended. No new matter is added by this amendment.

In regard to item (4), as indicated above, claims 16 and 17 (subparts (b)(4)) have been corrected to recite DPA1*0202-D**P**B1*0501 rather than "DPA1*0202-D**Q**B1*0501". No new matter is added by these corrections that are not intended to, and do not, narrow the scope of the claims.

In light of the above considerations, Applicants request that the rejections under 35 U.S.C. §112, first paragraph, be withdrawn.

35 U.S.C. §112, second paragraph, rejection

Claims 16 and 17 stand rejected as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter that Applicants regard as the invention.

From the comments on page 7, paragraph 12, of the Office Action, Applicants understand the Examiner's position to be that the recitation of "if" in claims 16 and 17 is indefinite and ambiguous and, as a result, one of ordinary skill in the art would not be appraised of the metes and bounds of the claimed invention. Applicants respectfully disagree with this position.

The test for indefiniteness is whether one of ordinary skill in the art would understand the metes and bounds of the claim when read in light of the specification and in the context of the prior art. Thus, claim language cannot be analyzed in a vacuum but must be interpreted in light of the specification, the teachings of the prior art, and the reasonable interpretation given by one of ordinary skill. Furthermore, it is not proper to reject a claim solely because of the type of language used to define the invention. All that

is required by the second paragraph of section 112 is a reasonable degree of precision and particularity. Accordingly, conditional expressions, such as the "if. . .then" steps set forth in claims 16 and 17, are not *per se* unacceptable. They are permitted so long as they present no ambiguity or uncertainty with respect to the scope and clarity of the claim. In fact, such conditional language is routinely used in claims drawn to chemical compounds defined in terms of a formula and substituents to expressly exclude or include specific embodiments (e.g., if R_1 is hydrogen, then R_2 is methyl or ethyl).

In the instant claims, one of ordinary skill in the art would readily understand that an essential feature of the instant claims is a selection step. For example, a method in which a subject is (a) identified as having a DQA1*0102-DQB1*0602 HLA class II molecule and (b) is treated with a peptide selected from the group consisting of SEQ ID NO: 1, 5, 7, 9, 10, 21, and 23 (as set forth in claim 16 (b)(1)) would be covered by the claim. On the other hand, if the same subject is identified as having that particular HLA class II molecule and then is treated with a <u>different</u> antigenic peptide (i.e., distinct from those of SEQ ID NO: 1, 5, 7, 9, 10, 21, and 23), the method would clearly be outside the scope of the claim (unless the subject falls within the claim by virtue of meeting one of the other criteria (b)(2)- (8), of course).

Accordingly, Applicants fail to see how the mere recitation of conditional language automatically gives rise to lack of clarity and submit that the burden is on the Examiner to demonstrate how confusion would arise as to the metes and bounds of the pending claims.

Notwithstanding these considerations, in the interest of expediting prosecution of the instant application, Applicants have replaced the term "if" with the term "when". These amendments are not intended to, and do not, narrow the scope of the claims.

In light of the above factors, Applicants request withdrawal of the rejection under 35 U.S.C. §112, second paragraph.

CONCLUSION

For the reasons set forth above, Applicants maintain that the pending claims patentably define the invention. Applicants request that the Examiner reconsider the rejections as set forth in the Office Action, and permit the pending claims to pass to allowance.

Attached is a marked-up version of the changes being made by the current amendment.

If the Examiner would like to discuss any of the issues raised in the Office Action, Applicants' undersigned representative can be reached at the telephone number listed below.

Applicants enclose a request for an automatic extension of time and a check in payment of the extension of time. Please charge any other fees or make any credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: 9/5/02

Stuart Macphail, Ph.D.
Reg. No. 44,217

Fish & Richardson P.C.
45 Rockefeller Plaza, Suite 2800
New York, New York 10111
Telephone: (212) 765-5070
Facsimile: (212) 258-2291

Version with markings to show changes made

In the specification:

On page 1, after the title ("PEPTIDE-BASED IMMUNOTHERAPEUTIC AGENT"), please insert:

--This application claims priority of International Application No. PCT/JP97/04129, filed November 12, 1997, which claims priority of Japanese Application No. 8/302053, filed November 13, 1996.--

The paragraph beginning at page 2, line 16, has been amended as follows:

-- Hyposensitization, a method for treating allergic diseases, comprises administering a small amount of antigen (for example, an antigen extracted from cryptomeria pollen or mites) to an allergy patient, and increasing the dosage gradually. The success of hyposensitization is attributed to the decreased response of allergen-specific T cells. Presumedly, hyposensitization causes T-cell tolerance (T cell anergy), and, as a result, cytokine, which is important for developing an allergic cascade, is not produced. Studies on allergies have focused on the allergen-specific immunoreaction in the early stage, especially on the mechanisms for controlling T-cell response to allergy. An allergic response to an exogenous antigen including an allergen is initiated depending on antigen-presenting cells in the immune system. Antigen-presenting cells, including B cells, macrophages, and dendritic cells, incorporate exogenous antigens, fragment the exogenous antigens into antigen peptides (T-cell epitope peptides), and express the fragmented antigens on the cell surface together with MHC class II (HLA class II for a human) to present an antigen to antigen-specific CD4 positive helper T cells (Th cells).--

The paragraph beginning at page 3, line 22, has been amended as follows:

-- Th cells that receive the antigenic information restricted by HLA class II molecules through T-cell receptors (TCR) are activated and secrete various cytokines to proliferate by themselves and differentiate B cells into plasma cells, thereby inducing

antibody production. At this time, the second signal (costimulatory signal), which is mediated by molecules other than TCR, is necessary to activate T cells. In contrast, without this signal, immunological tolerance of Th cells to an antigen is induced (June, C. et al.: Immunol Today, 15: 321, 1994).--

The paragraph beginning at page 4, line 5, has been amended as follows:

-- The decrease of T-cell response to an allergen is related to the success of hyposensitization. For example, the T-cell response in vitro to ambrosia allergen "Amb a 1" in a patient suffering from an ambrosia allergy who had undergone effective hyposensitization for ten years was dramatically decreased compared to [the] an untreated patient. Similarly, in a patient allergic to feline epidermis antigen "Fel d 1," T-cell response specific to Fel d 1 was obviously decreased, as hyposensitization showed effects. This decrease corresponded to the decrease of sensitivity in the skin test. Furthermore, IgG and IgE antibodies specific to Fel d 1 remained at a constant level during the treatment. These results indicated that a therapeutic agent for an allergy directly targeting antigen-specific T cells could be prepared.--

The paragraph beginning at page 7, line 20, has been amended as follows:

--Furthermore, specific T-cell epitopes [responding] binding to specific HLA class II molecules can be detected by the method of the present invention. The present inventors considered using the specific T-cell epitopes as a reagent for typing the patient's HLA class II molecules and completed the present invention. The reagent for typing the HLA class II molecules can be effectively used for selecting a peptide-based immunotherapeutic agent effective for individual patients.--

The paragraph beginning at page 8, line 7, has been amended as follows:

--["Recognize" means to activate T cells.] Epitopes that are "recognized" are epitopes that activate T cells. Whether T cells are activated or not can be observed by the production of cytokines, such as IL-2, IL-4 and IFN-γ or by DNA synthesis.--

The paragraph beginning at page 8, line 16, has been amended as follows:

--"Linkage disequilibrium" means the correlation found among different genes when alleles of different HLA loci are [recognized] present in a single chromosome or a haplotype with higher frequency than expected by chance. Linkage disequilibrium is quantified by the difference between the expected and observed values (Δ).--

The paragraph beginning at page 9, line 2, has been amended as follows:

--In the present invention, T-cell epitopes in an allergen molecule can be mapped by, for example, culturing peripheral blood lymphocytes, T-cell lines, or T-cell clones derived from a patient sensitive to a specific allergen, together with antigen-presenting cells and an overlapping peptide composed of about 15 to 30 amino acid residues (in which the overlapping portion is about 5 to 10 residues) which covers the whole primary structure of said allergen, determining T-cell response to these peptides by measuring the amount of [^{3}H]thymidine uptake (response by cell proliferation), and identifying the peptide to which T-cells responded. The exact epitope sites can be identified by synthesizing deletion variant peptides by [subsequently] deleting amino or carboxyl terminal amino acid residues of antigen peptides and monitoring the change of T-cell response to these variant peptides. Alternatively, when more than two peptides containing overlapping regions produce T-cell responses, the exact epitope sites can be identified by synthesizing new T-cell epitope peptides containing a part or all of the overlapping regions, and monitoring the change of T-cell response. The antigen peptide of the present invention preferably contains at least seven amino acid residues.--

The paragraph beginning at page 10, line 4, has been amended as follows:

--An antigen peptide of the present invention [has in vitro proliferation activity on peripheral blood lymphocytes] induces proliferation in vitro in peripheral blood lymphocytes, T-cell lines or T-cell clones derived from an individual allergy patient having HLA class II molecules restricting said peptide. An antigen peptide of the invention does not react with an IgE antibody of a patient sensitive to the allergen from which said peptide is derived. The antigen peptide of the present invention can induce antigen-specific T-cell anergy by the administration of the antigen peptide and thereafter can induce immunological tolerance at any time when challenge [of] with a recombinant or natural allergen derived from said antigenic peptide is made. Furthermore, once an antigen peptide of the present invention is administered to an individual sensitized by an allergen, [thereafter immunological tolerance can be induced at any time when the challenge of said allergen is made] immunological tolerance in the individual can be induced at any time thereafter by challenging with said allergen. These facts indicate that the antigenic peptide of the present invention induces an antigen-specific immunological tolerance in vitro and is useful for peptide-based immunotherapy of an allergy patient.--

The paragraph beginning at page 11, line 18, has been amended as follows:

-- At present, major allergens of cryptomeria pollen allergen, Cry j 1 and Cry j 2, have been isolated and purified. cDNAs of both allergens have been isolated, and their estimated primary structures have been disclosed (International patent application published in Japan Nos. Hei 8-502163 and Hei 8-505284). T-cell epitope sites in the Cry j 1 molecule were identified based on the [molecules] molecule's primary structure. A therapeutic composition for cryptomeria pollen allergy, composed of a peptide containing the epitope site as an effective ingredient, has been disclosed (International patent application published in Japan No. Hei 8-502163). It was reported that more than 90% of patients suffering from a cryptomeria pollen allergy have IgE antibodies specific to Cry j 1 and to Cry j 2; the remaining 10% of patients have IgE [antigen] antibody specific to either Cry j 1 or Cry j 2 (Hashimoto, M et al.: Clin. Exp. Allergy 44: 840-841, 1995).--

The paragraph beginning at page 12, line 6, has been amended as follows:

--Based on the above report, the present inventors thought that peptide-based immunotherapy by administering either Cry j 1 T-cell epitopes or Cry j 2 T-cell epitopes would not be sufficiently effective [enough]. The present inventors provided multiple epitope peptides with the minimum length effective for peptide-based immunotherapy to a cryptomeria pollen allergy caused by antigen peptides presented by HLA-DPB1*0501. HLA-DPB1*0501 is frequently [presented in a patient suffering from a cryptomeria pollen allergy and is derived from] present in patients suffering from cryptomeria pollen allergy induced by Cry j 1 and Cry J 2, and antigen peptides presented by different HLA class II molecules (DR, DQ or DP) (Japanese Patent Application No. Hei 8-80702).--

The paragraph beginning at page 12, line 17, has been amended as follows:

-- This multiple epitope peptide can be expected to [enhance the] have enhanced effectiveness in allergy patients but is ineffective for patients who do not have HLA molecules restricting an antigen peptide composed of said epitope peptides. An antigen peptide compatible with an individual HLA type should be administered to the individual for effective peptide-based immunotherapy.--

The paragraph beginning at page 12, line 23, has been amended as follows:

--Examples of combinations of the antigen peptides with types of HLA class II restriction molecules in [a patient] patients suffering from a cryptomeria pollen allergy are given below. Specific examples of HLA class II molecules and their binding partner antigen peptides include:--

The paragraph beginning at page 13, line 6, has been amended as follows:

--2) DRB4*0101 binds to antigen peptides p191-205 (SEQ ID NO: 7) derived from Cry j 1 and antigen peptides p16-30 (SEQ ID NO: 12) and p186-200 (SEQ ID NO: 18) derived from Cry j 2,--

The paragraph beginning at page 13, line 9, has been amended as follows:

--3) DQA1*0102-DQB1*0602 binds to antigen peptides p16-30 (SEQ ID NO: 1), p146-160 (SEQ ID NO:5), p191-205 (SEQ ID NO: 7), p251-265 (SEQ ID NO: 9), and p326-340 (SEQ ID NO: 10) derived from Cry j 1 and antigen peptides p326-340 (SEQ ID NO: 21), [and] p341-355 (SEQ ID NO: 23), and p346-360 (SEQ ID NO: 142) derived from Cry j 2,--

The paragraph beginning at page 13, line 14, has been amended as follows:

--4) DPA1*0101-DPB1*0501 binds to antigen peptides p61-75 (SEQ ID NO: 2) and [221] 211-225 (SEQ ID NO: 8) derived from Cry j 1 and antigen peptide p76-90 (SEQ ID NO: 15) derived from Cry j 2,--

The paragraph beginning at page 14, line 9, has been amended as follows:

--It has been conventionally hypothesized that there is a bias [for restriction molecules in the level of HLA class II locus depending on the types of antigens] in the use of HLA class II (at the locus level) molecules that is determined by the antigen. The above studies revealed that, in principle, all DR, DQ, and DP molecules are used as restriction molecules presenting antigen peptides derived from Cry j 1 or Cry j 2, without bias.--

The paragraph beginning at page 16, line 9, has been amended as follows:

--Specifically, HLA class II molecules of an allergic patient and a healthy subject can be typed as follows. Amino acid motifs of antigen peptides binding to each molecule vary depending on the HLA class II types due to their high polymorphism. HLA class II molecules of a patient and a healthy person can thus be typed by labeling antigen peptides [with] having different binding motifs and detecting the specific binding to HLA class II molecules. Antigen peptides can be labeled by binding a known label, such as a radioisotope, an enzyme, a fluorescent label, or a luminescent label, to an amino acid residue (for example a tyrosine residue) other than the HLA anchor amino acid residues of the antigen peptides. Alternatively, biotinylated antigen peptides are detected with [streptoavidin] streptavidin (or avidin) bound to the above label. An allergic patient can

be diagnosed by culturing peripheral blood lymphocytes of the subject in the presence of various antigen peptides derived from the allergen and monitoring T-cell response by, for example, adding [^{3}H]thymidine to the culture medium and measuring the amount of [^{3}H]thymidine uptake. Moreover, if T-cell response can be found in a subject (an allergic-response-positive patient), the type of the subject's HLA class II molecules restricting the antigen peptides that induced the T-cell response can be identified as [the subject's type of HLA class II susceptible to said allergen] the HLA class II type endowing susceptibility to said allergen in the subject. --

The paragraph beginning at page 17, line 5, has been amended as follows:

--The correlation between the patient's HLA class II type and the antigen peptides identified by this method can be used to study the [roll] role of each HLA class II type in the onset of allergy or to select antigen peptides to be used in a peptide-based immunotherapeutic agent for the allergic patient.--

The paragraph beginning at page 17, line 10, has been amended as follows:

-- A peptide-based immunotherapeutic agent for a particular allergic patient whose HLA class II molecule type has been identified can be prepared by selecting an antigen peptide compatible with the HLA type of said patient, measuring the response to the peptide to proliferate peripheral blood lymphocytes derived from the patient, and comparing the level of the response of the peptide. For example, the haplotypes of HLA class I and class II of patient PB suffering from cryptomeria pollen allergy described in Example 6 are: A2/24-B39/55-Cw7/w3-DRB1*1501/0901-DRB4*0101-DRB5*0101, [and] DQA1*0102/0301-DQB1*0602/0303, and [-]DP[B]A1*0101/0101-DPB1*0501/0201. When antigen peptides to be used for peptide-based immunotherapy for said patient are selected, the antigen peptides p211-225 (SEQ ID NO: 8) presented by DPA1*0101-DPB1*0501, p106-120 (SEQ ID NO: 3) presented by DBR5*0101, p191-205 (SEQ ID NO: 7) or p251-265 (SEQ IN NO: 9) presented by DQA1*0102-DQB1*0602 should be selected in Cry j 1; in Cry j 2, p76-90 (SEQ ID NO: 15) presented by DPA1*0101-DPB1*0501, p186-200 (SEQ ID NO: 18) presented by DRB4*0101, and

p66-80 (SEQ ID NO: 14) presented by DRB5*0101 should be selected. Before peptide-based immunotherapy is effected using these antigen peptides, the response to these antigen peptides to proliferate peripheral blood lymphocytes derived from the patient should be measured to select the antigen peptides exhibiting a relatively high proliferation activity, which antigen is to be used for peptide-based immunotherapy.--

The paragraph beginning at page 18, line 7, has been amended as follows:

--In order to improve solubility, therapeutic or prophylactic effects, and stability of the effects, the antigen peptide of the present invention can be modified by substituting, deleting, or adding amino acid residues other than the HLA anchors without spoiling their function. A certain amino acid can be suitably substituted with Ala, Ser, Glu, or methyl amino acids, but substituent amino acids are not limited thereto. Cys residue forms a dimer through a disulfide bond and functions as a multi-binder. Therefore, immunization with a peptide containing a Cys residue may cause recognition of sites which are not originally involved in antigenicity and thereby create new epitopes. In this case, a Cys residue can be substituted with Ala, Ser, Thr, Leu, or Glu. It may also be substituted by a D amino acid or a non-natural amino acid. A vector capable of expression [as a polypeptide, a peptide with] <u>of a polypeptide with a peptide composed of</u> a histidine polymer (for example, a histidine hexamer) at its N- or C-terminus[.] has been developed. The expression product can be purified by affinity chromatography using a nickel chelating column even in the presence of a denaturant. Such an embodiment is also included in the present invention.--

The paragraph beginning at page 19, line 6, has been amended as follows:

--Figure 1 shows the overlapping peptides [between Cry j 1 and the patient's T-cell epitope sites] <u>of Cry j 1 containing epitopes recognized by patient's T-cells</u>. In the figure, □ indicates $2 \leq SI < 5$ and ▫ $5 \leq SI$. T-cell clones were prepared from PB and PJ.--

The paragraph beginning at page 19, line 9, has been amended as follows:

--Figure 2 shows the overlapping peptides [between Cry j 2 and the patient's T-cell epitope sites] of Cry j 2 containing epitopes recognized by patient's T-cells. In the figure, □ indicates 2≤SI<5 and ▣ 5≤SI. T-cell clones were prepared from PB, PC and PR.--

The paragraph beginning at page 19, line 13, has been amended as follows:

--Figure 3 shows the epitope sites recognized by T-cell clones which recognize Cry j 1, the molecules restricting said clones, the production of lymphokines by said clones, and Th types of said clones. In the figure, Th2 stands for IL-4/IFN-γ>10, Th1 for IFN-γ/IL-4>10, and Th0 for [the] a level intermediate therebetween.--

The paragraph beginning at page 19, line 18, has been amended as follows:

-- Figure 4 shows the epitope sites recognized by T-cell clones which recognize Cry j 1, the restriction molecules of said clones, the production of lymphokines by said clones, and Th types of said clones. In the figure, Th2 stands for IL-4/IFN-γ>10, Th1 for IFN-γ/IL-4>10, Th0 for [the] a level intermediate therebetween, and Thp for no lymphokine production.--

The paragraph beginning at page 19, line 24, has been amended as follows:

--Figure 5 shows the immune responses of CB6F1 [mouse] mice to Cry j 2 when the antigen peptide p66-80 of Cry j 2 was administered to the [mouse] mice.--

The paragraph beginning at page 20, line 1, has been amended as follows:

--Figure 6 shows the immune responses of CB6F1 [mouse] mice to Cry j 2 when the antigen peptide p186-200 of Cry j 2 was administered to the [mouse] mice.--

The paragraph beginning at page 21, line 18, has been amended as follows:

--When [the] activated T cells appeared, T-cell lines specifically recognizing Cry j 1 or Cry j 2 were established by replacing the medium with RPMI-1640 medium with 200 U/ml of IL-2 (Boehringer-Mannheim) and 15% human serum and culturing the cells

for an additional 14 days.--

The paragraph beginning at page 21, line 22, has been amended as follows:

--T-cell clones specifically recognizing Cry j 1 or Cry j 2 were established as follows. When the activated T cells appeared, T cells were spread in a 10-cm culture dish and selected one-by-one using a micropipet. Separately, the same nonactivated cells transfected with EB virus were treated with mitomycin C (Kyowa Hakko Kogyo) and inoculated into each well of a 96-well microculture plate [to] at 1 x 10^5 cells/well. The above activated T cells were transferred to the 96-well plate, one cell per well. An additional 50μg/ml of Cry j 1 or 2 to 10μg/ml of Cry j 2 was added to each well and cultured for 7 days for challenge. The challenge at an interval of 7 days was repeated two or three times to establish T-cell clones.--

The paragraph beginning at page 22, line 8, has been amended as follows:

--Peripheral blood lymphocytes derived from 18 patients suffering from cryptomeria pollen allergy were challenged by Cry j 1 or Cry j 2 to establish T-cell lines specifically recognizing Cry j 1 or Cry j 2 for each individual patient. 5 x 10^4 cells of self-derived B-cell line treated with mitomycin C, 2μM of overlapping peptides, and 2 x 10^4 cells of the T-cell line were cultured in RPMI-1640 medium supplemented with 0.2 ml of 15% serum in a 96-well microplate for two days. 0.5μCi of [^{3}H]thymidine was added, and the culture medium was cultured for an additional 18 hours. The cells were collected in a glass filter with a cell harvester, and uptake of [^{3}H]thymidine was measured with a liquid scintillation counter. T cells capable of recognizing antigenic information of Cry j 1 or Cry j 2 as well as HLA class II molecules [proliferate and took [^{3}H]thymidine into the cells] proliferated and incorporated [^{3}H]thymidine. Cells exhibiting a Stimulation Index of 2 or higher were [recognized as] considered to have recognized the relevant added antigen peptides.--

The paragraph beginning at page 22, line 23, has been amended as follows:

--[In T-cell epitope sites identified using T-cell lines recognizing Cry j 1, the] The

number of T-cell epitope sites of Cry j 1 recognized by each patient was 9.8±3.0 on average and ranged from 4≤15 epitopes. Using T-cell lines recognizing Cry j 2, the number of T-cell epitope sites recognized by each patient was 8.7±3.3 on average and ranged from 2≤13 epitopes. Since Cry j 1 is composed of 353 amino acids (International patent application published in Japan No. Hei 8-502163) and Cry j 2 is composed of 379 amino acids (JP-A No. Hei 8-47392), the above results mean that about 2.3 to 2.8 T-cell epitope sites exist per 100 amino acid residues. Each patient has different HLA class II types and therefore recognizes different T-cell epitopes depending on the HLA class II types. An epitope map was prepared by marking T-cell epitope sites on the Cry j 1 or Cry j 2 molecule T-cell epitope sites recognized by each patient on the Cry j 1 or Cry j 2 molecule. The results are shown in Figs. 1 and 2.--

The paragraph beginning at page 24, line 6, has been amended as follows:

--35 and 14 types of T-cell clones specifically recognizing Cry j 1 were established from the peripheral blood lymphocytes derived from PB and from PJ, respectively. Similarly, 31, 10, and 17 types of T-cell clones specifically recognizing Cry j 2 were established from the peripheral blood lymphocytes derived from PB, PC, and PR respectively. All of these T-cell clones were $CD3^+$, $CD4^+$, $CD8^-$, TCR-$\alpha\beta^+$, TCR-$\gamma\delta^-$, therefore, the restriction [cells were found to be] molecules were HLA class II molecules. Self-derived 5×10^4 B-cell lines treated with mitomycin C, 2μM of the overlapping peptides, and 2×10^4 T-cell clones were cultured in RPMI-1640 medium supplemented with 0.2 ml of 15% serum on a 96-well microplate for 2 days. After 0.5μCi of [^{3}H] thymidine was added, the cells were further cultured for 18 hours. The cells were collected in a glass filter by a cell harvester and uptake of [^{3}H]thymidine was measured using a liquid scintillation counter. T-cell epitopes recognized by each T-cell clone were identified by the above manipulation. Sixty-nine percent (34/49) of T-cell clones recognizing Cry j 1 proliferated in response to stimulation by the peptide containing T-cell epitopes and the antigen peptides were identified. Similarly, antigen peptides were identified among 69% (40/58) of T cell clones recognizing Cry j 2. T-cell clones specifically recognizing Cry j 1 recognized peptides p16-30, p61-75, p91-105, p106-120,

p146-160, p151-165, p191-205, p211-225, p251-265, p320-340, and p331-346. T-cell clones specifically recognizing Cry j 2 recognized peptides p16-30, p21-35, p36-50, p66-80, p76-90, p81-95, p151-165, p181-195, p186-200, p236-250, p321-335, p326-340, p336-350, p341-355, and p346-360. The results are summarized in Figures 1 and 2 (the histograms in the center).--

The paragraph beginning at page 25, line 8, has been amended as follows:

--Example 7 Identification of HLA class II restriction molecule[s in the locus level] loci--

The paragraph beginning at page 25, line 10, has been amended as follows:

--HLA class II restriction molecules were identified [in] at the locus level by adding monoclonal antibodies specifically reacting with HLA-class II-DR, HLA-class II-DQ, or HLA-class II-DP to the proliferation response system of T-cell clones established in Example 4 so as to inhibit T cell proliferation response.--

The paragraph beginning at page 25, line 25, has been amended as follows:

--Restriction molecules of each HLA class II type of T-cell clones whose restriction molecules were identified [in] at the locus level can be identified by using, as antigen-presenting cells, mouse L-cells transformed with the DR gene and B-cell lines [having the same haplotype as DQ or DP] that are homozygous at the DQ or DP loci.--

The paragraph beginning at page 27, line 8, has been amended as follows:

--Figures 3 and 4 show the production of IL-2, IL-4, and [IF-Nγ] IFN-γ and Th types of each clone. The number of T-cell clones recognizing Cry j 1 are [12 in Th2 cells, 1 in Th1 cells, 16 in Th0 cell,] 12 Th2 clones, 1 Th1 clone, and 16 Th0 clones. [thus] Thus the number of Th2 was larger than that of Th1. In contrast, the number of T-cell clones recognizing Cry j 2 are [10 in Th2 cells, 8 in Th1 cells, 8 in Th0 cells,] 10 Th2 clones, 8 Th1 clones, and 8 Th0 clones. [thus] Thus the number of Th1 was almost the same as that of Th2. [Comparing T-cell epitopes recognizing] In comparing T cell epitopes recognized by each T cell clone, the restriction molecules, and Th types, it was

found that each T-cell clone was different [in Th2, Th1, and Th0 types] with respect to Th type. For several T cell clones recognizing the same epitopes and same antigen-presenting molecules, both Th2 and Th1 cells were identified. These findings indicate that differentiation of T cells to Th2, Th1, or Th0 cells after stimulation by Cry j 1 or Cry j 2 is not determined by the combination of specific T-cell epitopes or specific restriction molecules. In other words, any peptide containing T-cell epitope sites can stimulate T cells and can be selected as peptides for use as a peptide-based immunotherapeutic agent.--

The paragraph beginning at page 27, line 26, has been amended as follows:

--Eight-week-old male CB6F1 mice were immunized with 10 μg of recombinant Cry j 2 (rCry j 2) together with an adjuvant (Imject Alum, PIERCE) three times every two weeks (ip). One week after the last immunization, splenocytes were prepared from three mice and combined. [5 x 10^6 splenocytes were cultured, together with 0.115μM of 74 types of the overlapping peptides consisting of 15 amino acid residues.] 0.115 μM each of 74 overlapping peptides consisting of 15 amino acid residues was cultured separately with 5 x 10^6 splenocytes in 0.2 ml of RPMI-1640 medium (10% FCS, 2 mM L-glutamine, 50 U/ml penicillin, and 50 μg/ml streptomycin) in each well of a 96-well plate (Falcon). As the control, the responses to PBS, 50 μg/ml of Cry j 1, 0.3 μg/ml of rCry j 2 were assessed. Each reagent was inoculated in three wells and cells were cultured at 37°C [under] in 5% CO_2 for three days. Pulse labeling was performed with 0.5μCi/well of [^{3}H]thymidine for the last 6 hours and the cells were collected in a glass filter using a cell harvester (Inoteck, Bertold Japan). After the cells were dried, uptake of [^{3}H]thymidine into the cells was measured with a liquid scintillation counter (TRI-CARB 4530, Packard Japan).--

The paragraph beginning at page 28, line 16, has been amended as follows:

--CB6F1 mice immunized with rCry j 2 showed a strong response to rCry j 2 antigen, but did not respond to another cryptomeria pollen major allergen Cry j 1, indicating that this system was antigen-specific reaction. [CB6F1 mice immunized with

rCry j 2 showed the remarkable responses to p66-80 (SEQ ID NO: 14) and p236-250 (SEQ ID NO: 48) among tested 90 kinds of overlapping peptides.] Of the 74 overlapping peptides tested, splenocytes from CB6F1 mice immunized with rCry j 2 showed remarkable responses to p66-80 (SEQ ID NO: 14) and p236-250 (SEQ ID NO: 19). These results indicated that p66-80 and p236-250 peptides [involved in the antigen presentation as a major T-cell epitope in CB6F1 mice] are presented as major epitopes of Cry j 2 in CB6F1 mice. In [a human] humans, p66-[180] 80 (SEQ ID NO: 14) and p236-250 (SEQ ID NO: [48] 19) are also major T cell epitope peptides. Thus, [a] CB6F1 [mouse] mice can be a useful model animal to evaluate the effectiveness of peptide compositions to be used in peptide-based immunotheraphy for cryptomeria pollen allergy.--

The paragraph beginning at page 29, line 4, has been amended as follows:

--Three mg of p66-80 peptide (SEQ ID NO: 14) dissolved in physiological saline was subcutaneously administered to an eight-week-old male mouse twice at an interval of 5 days. Similarly, the same volume (100 μl) of physiological saline was administered to mice of the control group. [Each of] Both the peptide-administered group and [the] a control group had eight mice. Five days after the second peptide administration, 50 μg of rCry j 2 mixed with an adjuvant, Imject Alum, was subcutaneously administered to all mice for immunization. One week after the immunization, splenocytes were prepared from each mouse. 5 x 10^6 splenocytes were cultured together with 3 μg/ml of rCry j 2 in 0.2 ml of RPMI medium (10% FCS, 2mM L-glutamine, 50 U/ml penicillin, and 50 μg/ml streptomycin) in each well of a 96-well plate (Falcon). As the control, the cells were cultured in the same medium containing no rCry j 2. Uptake of [^{3}H]thymidine was measured as described in Example 10.--

The paragraph beginning at page 29, line 19, has been amended as follows:

--When p66-80 (SEQ ID NO: 14) was subcutaneously administered to CB6F1 mice before antigen stimulation by rCry j 2, immune response of the T cells was significantly inhibited compared to the physiological saline-administered group ($p<0.01$)

(Figure 5). This result indicated that in the mouse model p66-80 (SEQ ID NO: 14) system showed a preventive effect in peptide-based immunotherapy for treating cryptomeria pollen allergy.--

The paragraph beginning at page 29, line 26, has been amended as follows:

--Example 12 In vivo immune response to antigen peptide p236-250 (peptide no. 48) (SEQ ID NO: [48] 19)--

The paragraph beginning at page 30, line 2, has been amended as follows:

--Three mg of p236-250 peptide (SEQ ID NO: [48] 19) dissolved in physiological saline was subcutaneously administered to a six-week-old male mouse twice at an interval of 5 days. As a control, the same volume (200 µl) of physiological saline was administered to mice in the same manner as above. [Each of] Both the peptide-administered group and the control group had eight mice. Five days after the second peptide administration, 50 µg of rCry j 2 mixed with adjuvant Imject Alum was subcutaneously administered to all mice. One week after the immunization, splenocytes were prepared from each mouse. 5×10^6 splenocytes were cultured together with 3 µg/ml of rCry j2 in 0.2 ml of RPMI medium (10% FCS, 2mM L-glutamine, 50 U/ml penicillin, and 50 µg/ml streptomycin) in each well of a 96-well plate (Falcon). As a control, the cells were cultured in the same medium containing no rCry j 2. Uptake of [^{3}H]thymidine was measured as described in Example 10.--

The paragraph beginning at page 30, line 17, has been amended as follows:

--When p236-250 (SEQ ID NO: [48] 19) was subcutaneously administered to CB6F1 mice before antigenic stimulation by rCry j 2, immune response of the T cells was significantly inhibited compared to the physiological saline- administered group ($p < 0.05$) (Figure 6). This result indicated that in the mouse model system p236-250 (SEQ ID NO: [48] 19) showed a preventive effect in peptide-based immunotherapy for treating cryptomeria pollen allergy [(Figure 6)].--

The paragraph beginning at page 31, line 2, has been amended as follows:

--According to the present invention, an antigen peptide that [matches] binds to a haplotype of HLA class II molecules of [each] an allergic patient can be used as a peptide-based immunotherapeutic agent for [the same] that patient. The present invention enables the optimal peptide-based immunotherapy for [each patient, thus,] individual patients. Thus the effectiveness of peptide-based immunotherapy is expected to be remarkably improved. Furthermore, the present invention provides a peptide-based immunotherapeutic agent effective for a patient who cannot be treated by peptide-based immunotherapy using major antigen peptides recognized in a specific patient population.--

In the claims:

Claims 16 and 17 have been amended as follows:

16. (Amended) A method for treating or preventing [allergy in a patient suffering from a] Japanese cedar pollen allergy in a subject in need thereof, the method comprising:

(a) identifying an HLA class II molecule expressed by the [patient] subject;

(b) selecting an antigenic peptide derived from Japanese cedar pollen allergen Cry j 1 or [Japanese cedar pollen allergen] Cry j 2, wherein the antigenic peptide binds to HLA class II molecule and wherein:

(1) [if]when the HLA class II molecule identified in step (a) is DQA1*0102-DQB1*0602, the antigenic peptide is selected from the group consisting of SEQ ID NO: 1, 5, 7, 9, 10, 21, 23, and [25]142;

(2) [if]when the HLA class II molecule identified in step (a) is DPA1*0101-DPB1*0501, the antigenic peptide is selected from the group consisting of SEQ ID NO: 2, 8, and 15;

(3) [if]when the HLA class II molecule identified in step (a) is DPA1*0101-DPB1*0201, the antigenic peptide is SEQ ID NO: 17;

(4) [if]when the HLA class II molecule identified in step (a) is DPA1*0202-[DQB1]DPB1*0501, the antigenic peptide is SEQ ID NO: 22;

(5) [if]when the HLA class II molecule identified in step (a) is DRB5*0101, the antigenic peptide is selected from the group consisting of SEQ ID NO: 3, 4, 14, and 19;

(6) [if]when the HLA class II molecule identified in step (a) is DRB1*0901, the antigenic peptide is selected from the group consisting of SEQ ID NO: 6, 7, 12, 16, and 20;

(7) [if]when the HLA class II molecule identified in step (a) is DRB4*0101, the antigenic peptide is selected from the group consisting of SEQ ID NO: 7, 12, and 18; and

(8) [if]when the HLA class II molecule identified in step (a) is DRB1*1501, the antigenic peptide is selected from the group consisting of SEQ ID NO: 13 and 19; and

(c) administering the selected antigenic peptide to [patient] the subject.

17. (Amended) A customized pharmaceutical composition for treating a [patient] subject suffering from a [cryptomeria] Japanese cedar pollen allergy, the composition comprising:

(a) an effective amount of an antigenic peptide derived from Japanese cedar pollen allergen Cry j 1 or [Japanese cedar pollen allergen] Cry j 2, wherein the antigenic peptide binds to an HLA class II molecule expressed by the [patient] subject and wherein:

(1) [if]when the [patient] subject expresses the HLA class II molecule DQA1*0102-DQB1*0602, the antigenic peptide is selected from the group consisting of SEQ ID NO: 1, 5, 7, 9, 10, 21, 23, and [25]142;

(2) [if]when the [patient] subject expresses the HLA class II molecule DPA1*0101-DPB1*0501, the antigenic peptide is selected from the group consisting of SEQ ID NO: 2, 8, and 15;

(3) [if]when the [patient] subject expresses the HLA class II molecule DPA1*0101-DPB1*0201, the antigenic peptide is SEQ ID NO: 17;

(4) [if]when the [patient] subject expresses the HLA class II molecule DPA1*0202--[DQB1]DPB1*0501, the antigenic peptide is SEQ ID NO: 22;

(5) [if]when the [patient] subject expresses the HLA class II molecule DRB5*0101, the antigenic peptide is selected from the group consisting of SEQ ID NO: 3, 4, 14, and 19;

(6) [if]when the [patient] subject expresses the HLA class II molecule DRB1*0901, the antigenic peptide is selected from the group consisting of SEQ ID NO: 6, 7, 12, 16, and 20;

(7) [if]when the [patient] subject expresses the HLA class II molecule DRB4*0101, the antigenic peptide is selected from the group consisting of SEQ ID NO: 7, 12, and 18; and

(8) [if]when the [patient] subject expresses the HLA class II molecule DRB1*1501, the antigenic peptide is selected from the group consisting of SEQ ID NO: 13 and 19; and

(b) a pharmaceutically acceptable diluent or carrier.

In the drawings:

In Fig. 1, please add an additional column indicating the SEQ ID NOs of the Cry j 1 peptides listed in the figure (see enclosed copy of Fig. 1 with the relevant SEQ ID NOs shown in red).

In the next to last line of Fig. 1, delete "tymidine" and insert in its place --thymidine--.

In Fig. 2, please add an additional column indicating the SEQ ID NOs of the Cry j 2 peptides listed in the figure (see enclosed copy of Fig. 2 with the relevant SEQ ID NOs shown in red).

In Fig. 2, please replace the lower case "a" at the end of the last amino acid sequence ("366-380 SGHVIPACKNLSPSa") with an upper case "A".